HUMAN GENOME SCIENCES
LEGAL DEPARTMENT

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FAX COVER SHEET

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TO: Jennifer Graser
United States Patent & Trademark Office

FAX NO.: (571) 273-0858

FROM: Marcy Rossell

Re: U.S. Application No. 10/068,956, filed February 11, 2002

Dear Ms. Graser

Per your telephone conversation with Joe Schuller today, attached is page 176 of the above-identified patent application.

If you experience any difficulty receiving this transmission, please contact Marcy Rossell (240) 314-4459.

10-14 days single clones are trypsinized and then seeded in 6-well petri dishes or 10 ml flasks using different concentrations of methotrexate (50 nM, 100 nM, 200 nM, 400 nM, 800 nM). Clones growing at the highest concentrations of methotrexate are then transferred to new 6-well plates containing even higher concentrations of methotrexate (1 μM, 2 μM, 5 μM, 10 μM, 20 μM). The same procedure is repeated until clones are obtained which grow at a concentration of 100 - 200 μM. Expression of PGRP-L is analyzed, for instance, by SDS-PAGE and Western blot or by reversed phase HPLC analysis.

Example 9: Construction of N-Terminal and/or C-Terminal Deletion Mutants

[0532] The following general approach may be used to clone a N-terminal or C-terminal deletion PGRP-L deletion mutant. Generally, two oligonucleotide primers of about 15-25 nucleotides are derived from the desired 5' and 3' positions of a polynucleotide of SEQ ID NO:1. The 5' and 3' positions of the primers are determined based on the desired PGRP-L polynucleotide fragment. An initiation and stop codon are added to the 5' and 3' primers respectively, if necessary, to express the PGRP-L polypeptide fragment encoded by the polynucleotide fragment. Preferred PGRP-L polynucleotide fragments are those encoding the N-terminal and C-terminal deletion mutants disclosed above in the "Polynucleotide and Polypeptide Fragments" section of the Specification.

[0533] Additional nucleotides containing restriction sites to facilitate cloning of the PGRP-L polynucleotide fragment in a desired vector may also be added to the 5' and 3' primer sequences. The PGRP-L polynucleotide fragment is amplified from genomic DNA or from the deposited cDNA clone using the appropriate PCR oligonucleotide primers and conditions discussed herein or known in the art. The PGRP-L polypeptide fragments encoded by the PGRP-L polynucleotide fragments of the present invention may be expressed and purified in the same general manner as the full length polypeptides, although routine modifications may be necessary due to the differences in chemical and physical properties between a particular fragment and full length polypeptide.

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